

Research Article

Bacterial Leakage and Microgap along Implant-Abutment Connection in Three Different Implant Systems

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ABSTRACT:

A microgap between implant and abutment connection can act as a bacterial source and cause inflammation, even endanger Osseointegration and subsequently change clinical and histological parameters. The goal of this study was to evaluate the microgap and microbial leakage of implant-abutment connection in three different implant systems. In this experimental study, 28 implants in 3 groups (10 Zimmer with conical connection of 8 degrees, 10 Dentium with conical connection of 11 degrees, 8 Test implants with conical connection of 16 degrees) were used. Microleakage of *Escherichia coli* was assessed at intervals of 5, 24, 48 hours and 2 weeks. Microgap was measured at 4 random points by scanning electron microscope. Data were analysed by Spss version 22 and kruskal-Wallis, Mann-Whitney, Chi-square, Kaplan- Meier tests. ($\alpha=0.5$) Mean microgap was $4.8\mu\text{m}$ (± 2.2) in Zimmer group, $3.1\mu\text{m}$ (± 1.4) in Implantium group and $16.9\mu\text{m}$ (± 8.7) in test group. After 2 weeks from start of the study, 20 percent of Zimmer and Dentium implants and 25 percent of test implant showed microleakage. Microleakage between Zimmer and Dentium implants was not significant; however, there was a significant difference between test implant and other groups. Microbial leakage was observed in all three implant systems. Although; there were differences in microgap between three groups, Microbial leakage was not statistically significant.

Keywords: Dental implant, Bacteria, Microbial leakage, Microgap, Implant-abutment connection, Scanning electron microscope.

[I] NTRODUCTION

Dental implants have had a revolutionary effect on prosthetic treatments [1]. Long term success of dental implants is well proven and histological factors [2, 3]. Despite of tremendous success of dental implants, there have been reported

shortcomings related to mechanical and microbial factors [4-6]. In two piece implant systems, abutment is connected to the implant by mechanical means and therefore, there will be a gap between implant and abutment [7, 8]. This

microgap can vary between 1 to 50 μm depending on the implant system and the torque value [9, 10]. Broggini et al demonstrated that presence of microgap between implant and abutment was associated with peri-implant inflammation and bone loss [11]. It is important to note that people who have been treated for periodontal disease, have a higher risk of peri-implantitis [12]. Pathogens in oral cavity must be reduced by proper plaque control before each surgical phase [12]. Microgap can act as a microbial source and subsequently endanger mucosal seal. Changes in clinical and microbiological parameters will cause spread of periodontal disease and endangers Osseointegration [11, 15-18]. Alkan et al reported that microgap along implant-abutment interface may cause undesirable stress distribution on connection [19]. Dental implants may have problems relating to screw loosening and breakage [20]. Failures related to screw breakage may be due to insufficient fitness of implant-abutment connection [7, 8 & 21]. Prevention of microbial leakage at implant-abutment connection is a big problem in fabrication of two piece implants to reduce inflammatory reactions and to increase bone stability around the neck of the implant. Different implant systems have different connections; the most common are internal, where a part of the abutment is inserted in the body of the implant, and the external, where the abutment is placed above the implant [12]. Research led by Larrucea Verdugo et al. revealed that Morse taper connection implants show lower levels of microleakage than external connection implants [13]. Furthermore, the internal conical implant-abutment connection is considered mechanically more stable and tighter than flat to flat or tube-in tube connections [14]. The degree of conical implant-abutment connection is different. Some types of implants have conical connection of 8 degrees such as Zimmer and some have conical connection of 11 degrees such as Implantium. It is not clear which connection

will be best to reduce microleakage. In this study we fabricated an implant by CNC machines with increasing conical connection up to 16 degrees and called it test implant [Figure-1]. Lack of standard results in relation to microgap and its effect on bacterial colonization has made it difficult to provide information about leakage between inner and outer parts of implant-abutment connection. The goal of this study was to assess the microgap and microleakage in three different implant systems and compare microleakage and microgap of our test implant with increased conical connection and compare it to two standard implant namely Zimmer and Implantium which have two different connection.

[II] MATERIALS AND METHODS

In this experimental study, 3 different groups for a total of 28 implants were scheduled. 10 Zimmer implants with platform of 4mm and conical connection of 8 degrees (Swiss plus, USA), 10 Implantium implants with platform of 4.3mm and conical connection of 11 degrees (Implantium, Dentium, Seoul Korea) and 8 Test implants with platform of 4.3mm and conical connection of 16 degrees (Mobtakerane Iran, Isfahan, Iran) were utilized.

2.1. Sterilization of implants and abutments before testing

In order to avoid external contamination, all implants, abutments and required instruments were autoclaved in the standard condition (121° c and 15 psi). All the experimental procedures were performed in sterile conditions in a proper microbiological hood under vertical laminar flow.

2.2. Microbial leakage test

For microbiological test, pure bacterial culture of *Escherichia coli* (E-coli) was used. E-coli are gram negative facultative anaerobic bacteria which has a diameter of 1.1 to 1.5 micron with a length of 2 to 6 micron. These bacteria have high ability to move and are widely used in similar studies on implants. To prepare a bacterial suspension, E-coli was cultured on a blood agar

(Mark Darmstadt, Germany) and kept at 37 °c for 24 hours. Then it was diluted in TBS (Difco, Lawrence, Kan) until standard density of 0.5 McFarland (1.5×10^8 CFU/ml) was reached. Each implant was hold by a haemostat and 1 microliter of bacterial suspension was placed in to internal space of the implant by a micropipette (Eppendorf, Hamburg, Germany). Subsequently abutments were screwed on to the implants with closing torque of 35 N/cm². Samples were then placed in to Eppendorf micro tubes, TSB culture was added to the tube, in such a way that the culture would stand above implant-abutment connection, and lower than the abutment screw [figure2]. To ensure sterility of external surface of the samples during this process; a test sample was cultured when TBS was added and placed in to the incubator at 37 °c. If the collected culture was positive, the contaminated sample was sterilized and retested again. Samples in each group were numbered and were transferred in to an incubator at 37 °c temperature. 0.1 ml of culture around dental implant were taken by a sampler and cultured on a blood agar at 5, 25, 48 hours and 14 days interval. All stage of procedures was done with sterile gloves under hood (Jal Tajhiz, Tehran, Iran) which was sterilized by ultraviolet light for one hour prior to the start of the experiment. All microbiological experiments were performed by a microbiologist whom was uninformed about the groups.

2.3. Microgap determination in implant-abutment connection by means of a scanning electron microscope

After completion of microbiological experiments, all samples were placed in to an ultrasonic cleaner for 30 minutes and then were autoclaved for elimination of contamination between implant and abutment. Microgap was measured at four random points by means of a scanning electron microscope (SEM) with voltage of 15 kV [Figure 3-a, b & c]. Results were analyzed by kruskal-Wallis, Mann—Whitney, Chi-square and Kaplan-Meier test and by Spss software version 22 ($\alpha=0.5$) [chart 1].

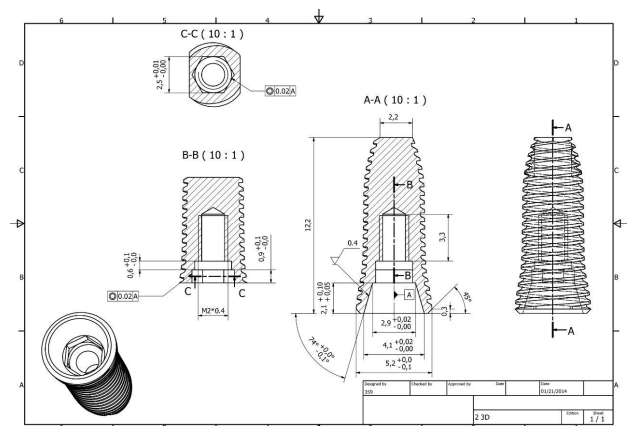


Fig: 1. Test implant with platform of 4.3mm and conical connection of 16 degrees



Fig: 2. Sample in TSB culture

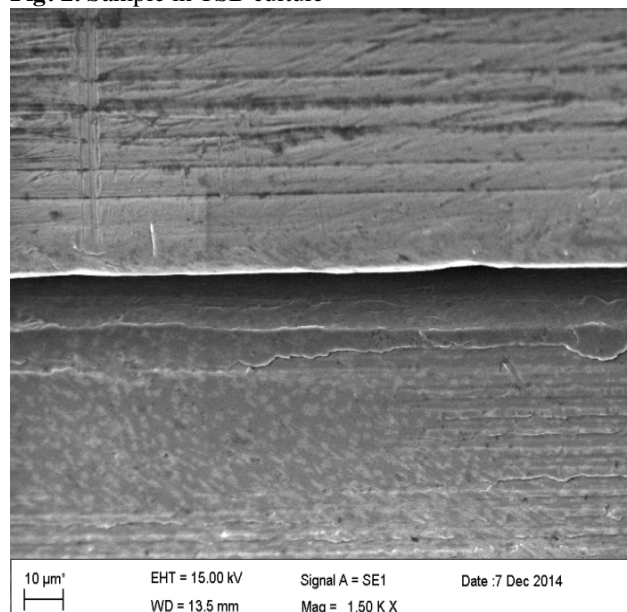


Fig: 3-a. scanning electron microscope view of Zimmer implant

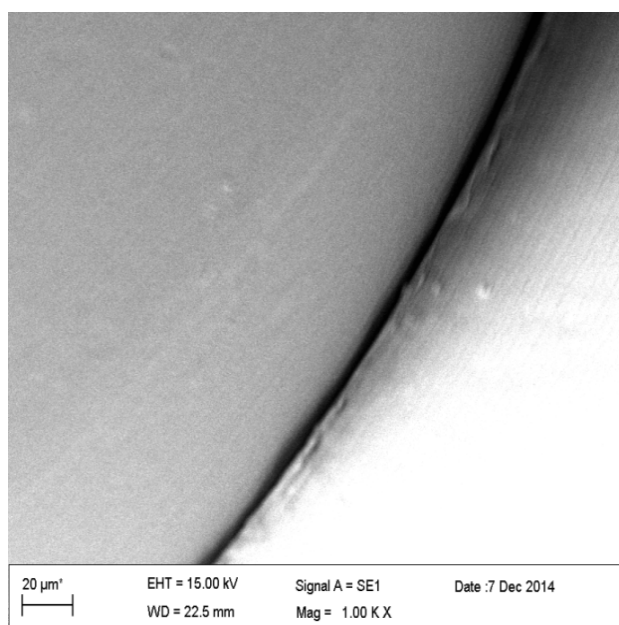


Fig: 3-b. scanning electron microscope view of implantium implant

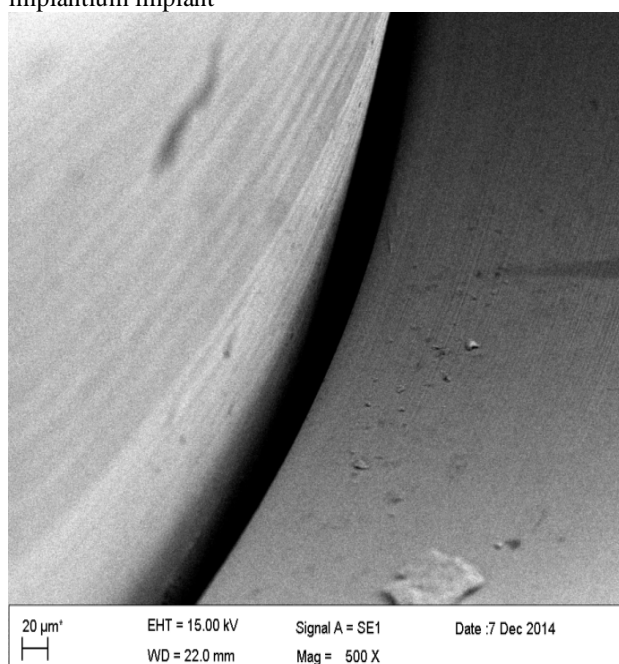


Fig: 3-c. scanning electron microscope view of test implant

[III] RESULTS

Mean microgap was $4.8\mu\text{m}$ (± 2.2) in Zimmer group, $3.1\mu\text{m}$ (± 1.4) in Implantium group and $16.9\mu\text{m}$ (± 8.7) in test group ([Table 1]).

Because of non-homogeneity between groups, kruskal-Wallis test was used instead of one way analysis of variance. This test indicated that there

was a statistical difference between three groups ($p < 0.001$). Subsequently, Mann-Whitney test indicated that there was no statistical difference between Zimmer and Implantium group ($p = 0.063$), but there was a statistical difference between test implant and two other groups ($p < 0.001$). In microbial leakage tests, one of the implants from the Implantium group and 2 test implants, showed micro leakage during first 5 hours. Micro leakage was observed in one implant from Implantium group after 24 hours, one implant in Zimmer group after 48 hours, and one other implant from Zimmer group after 2 weeks [Figure 2]. Chi-square test showed no difference in micro-leakage between three groups after 2 weeks. Kaplan-Meier test also did not show any difference in micro leakage between groups ($p = 0.932$).

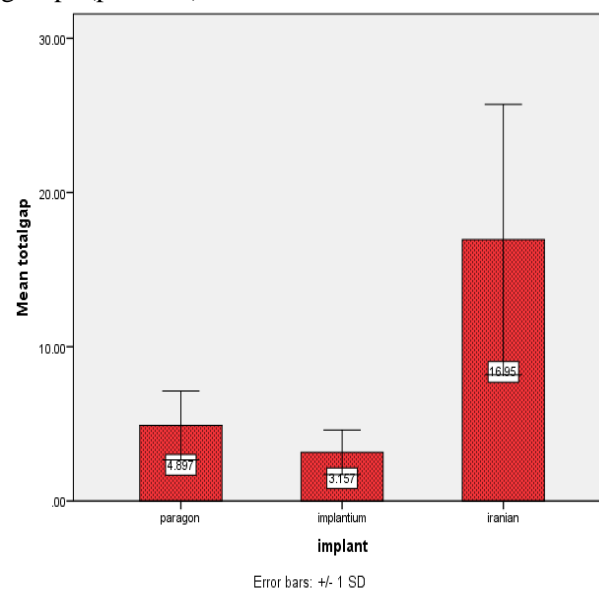


Chart: 1. Microgap values of implant abutment interface (μm)

[IV] DISCUSSION

The main goal of this study was to evaluate the microgap and microleakage in different implant-abutment connection. The microgap between implant and abutment in two piece implants can cause colonization of bacteria and inflammation in tissues around implants and subsequently bone loss. Additionally, microgap can cause mechanical problems such as abutment screw

loosening. The size of microgap in the implant-abutment connection varied between 1 to 50 μm according to different studies.

In the study by Rismanchian et al. [22] the size of the microgap was reported to be between 7 to 74 μm depending on the abutment type. Fernandez et al. [20] reported the size of the microgap in the range of 0.73 to 11.30 μm depending on the type of abutment. Tesuge et al. [23] have reported mean microgap of 3.2 to 5.6 μm . Also, in a study By Scarano et al. [24] the size of the microgap was 60 μm in screw type and 40 μm in cemented type prosthesis. Piattelli et al. [4] have reported microgap of 2-7 μm in screw type and 7 μm in cemented type prosthesis. In a study by Jansen et al. [10] the amount of the microgap was less than 10 μm in all tested implants. In this study, mean microgap was 4.8 μm in Zimmer group, 3.1 μm in Implantium group and 16.9 μm in tested implant group. Mean microgap in Zimmer and Implantium groups was similar to some previous studies [10, 20 & 23]. Although microgap in Zimmer group was bigger than Implantium, but microleakage was occurred later in Zimmer group. The bacterium used was the *Escherichia coli*, a gram-negative, motile, and facultative anaerobic bacterium. It is an opportunistic human pathogen occasionally associated with implant failure [12]. Already used in microbial leakage dental implant studies [10, 24-26]. In addition if we had measured more points in implant-abutment connection area with SEM and had a larger sample size, we might have had different amount for microgap, although this difference was not statistically significant. Highest mean of microgap seen in the test implant may be due to the fact that this implant is still in its primary stages and needs further investigations and improvement in machining precision between its parts.

Harder et al. [14] studied microleakage in two systems of Astra tech and Ankylose, both of which have internal connection of 11 degrees. Their results indicated only one Astra tech implant showed endotoxin contamination after 7

days, whereas all Ankylose implants showed contamination after 5 minutes. Faria et al. [28] compared microleakage of *E. coli* in three different connections between abutments and implants (External hex, internal hex and Morse taper). They observed similar bacterial leakage in all three types of connections. Piattelli et al. [4] and Scarano et al. [27] studied microleakage in two systems of screw type and cemented type. In both studies microleakage was reported in screw types but no microleakage reported in cemented type. Jansen et al. [10] reported microleakage in all tested implant systems. Bajoghli et al. [29] evaluated the bacterial microleakage of implant-abutment connection area in three different implant systems (Zimmer, Implantium and Biohorizon) by the penetration method in different time intervals. They observed leakage in all three systems over time. Diabert et al. [30] did not observe any microleakage among 25 implants with locking tapered connection (Bicon, Bicon, Boston, EUA). This is in contrary to this study and most other studies. In our study, the evaluation period was up to two week but in Diabert study [30] evaluation period was up to 72 hours and this can explain the different result. Aloise et al. [31] studied two more taper implants systems (Bicone and Ankylose). They observed microleakage in 20 percent of both systems after 48 hours. In our study, microleakage was found in 20 percent of Zimmer and Implantium groups and in 25 percent of test implant group after 2 weeks. Our results are similar to Aloise et al. [31] study. Higher microleakage in test implants may be due to larger microgap between implant-abutment connection compare to other two groups. Our test implant had conical connection of 16 degrees. We were hoping to decrease the microleakage through increasing the degree of conical connection; however, there was no significant difference between groups. Differences in microgap and microleakage values in different studies indicated that further studies and

techniques are necessary to improve implant-abutment connection.

[V] CONCLUSION

Within limitation of this study it can be concluded that microleakage existed in all three implant systems. Although; there were differences in microgap between three groups, but Microbial leakage was not statistically significant.

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